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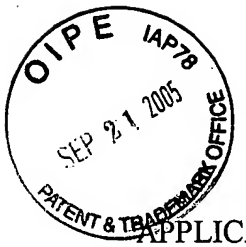

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	LINDA S. POWERS, ET AL.
FOR:	TAXONOMIC IDENTIFICATION OF PATHOGENIC MICROORGANISMS AND THEIR PROTEINS
SERIAL NO.:	10/706,547
FILING DATE:	11/12/2003
GROUP ART UNIT:	1641
EXAMINER:	MELANIE J. YU

BRIEF ON APPEAL

Applicants filed a Notice of Appeal on May 23, 2005, to the Board of Appeals from the final rejection of all claims remaining in the application. Requests for Three-Months Extension of Time to File Appeal Brief were filed on July 22, 2005, and August 19, 2005.

REAL PARTY IN INTEREST

The real parties in interest, Linda S. Powers; Walther R. Ellis, Jr.; and Christopher R. Lloyd are named in the caption of the brief.

RELATED APPEALS AND INTERFERENCES

There are no related appeals and interferences known to appellant, the appellant's legal representative, or assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

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STATUS OF CLAIMS

In the Final Official Action dated December 23, 2004, Claims 21-37 and 53 are pending in the application. Of the above Claims 24, 25 and 27-37 are withdrawn from consideration; Claims 1-20, 38-52, and 54-83 are canceled; Claims 21-23, 26 and 53 are currently amended and appealed.

CLAIM AMENDMENTS

What Is Claimed:

1. (Cancelled)

2. (Cancelled)

3. (Cancelled)

4. (Cancelled)

5. (Cancelled)

6. (Cancelled)

7. (Cancelled)

8. (Cancelled)

9. (Cancelled)

10. (Cancelled)

11. (Cancelled)

12. (Cancelled)

13. (Cancelled)

14. (Cancelled)

15. (Cancelled)

16. (Cancelled)

17. (Cancelled)

18. (Cancelled)

19. (Cancelled)

20. (Cancelled)

21. (Currently Amended) A method for ~~taxonomic~~ identification of a biological analyte comprising:

(a) exposing a solution containing the analyte to a ligand specific for the analyte of interest that has been covalently ~~tethered~~ bound directly to a photostable linker, said linker covalently tethered to a substrate surface wherein said photostable linker has a length of at least 6 Å, ~~to a substrate surface with a photostable linker at a distance of at least six Å for the capture of proteins~~;

(b) separating the bound analyte from the non-binding components of the solution containing the analyte by physical ~~separation,~~ separation of the substrate surface from the sample, washing or both; and

(c) interrogation of the ligand-tethered substrate surface for analyte binding by detection of the bound analyte.

22. (Original) The method of claim 21, wherein the biological analyte is selected from the group comprised of:

(a) proteinaceous toxins; and

(b) cytosolic proteins.

23. (Currently Amended) The method of claim 21, wherein the ligand is a peptide, ~~usually~~ comprised of three to twenty amino acids ~~long~~, specific for a proteinaceous toxin.

24. (Withdrawn) The method of claim 21, wherein the ligand is a peptide, usually three to twenty amino acids long, specific for a proteinaceous hormone.

25. (Withdrawn) The method of claim 21, wherein the ligand is a peptide, usually three to twenty amino acids long, specific for a cytosolic protein.

26. (Currently Amended) The method of claim 21, wherein the ligand is a peptide ~~that does not contain tryptophan or tyrosine~~ and detection of the captured analyte is accomplished through interrogation of the surface to detect ~~the~~ an intrinsic fluorescence of the ~~protein.~~ tryptophan and/or tyrosine residues present in the captured protein where said intrinsic fluorescence is detected between 300 and 400 nm upon excitation by ultraviolet light between 200 and 300 nm.

27. (Withdrawn) The method of claim 21, wherein the detection of the captured analyte is accomplished through the fluorescence of a reactive dye conjugate exposed to the protein before capture of the analyte by the tethered ligand surface.

28. (Withdrawn) The method of claim 21, wherein the detection of the captured analyte is accomplished through the fluorescence of a reactive dye conjugate exposed to the protein after capture of the analyte by the tethered ligand surface.

29. (Withdrawn) The method of claim 21, wherein the detection of the captured analyte is accomplished through the radioactivity of a reactive compound exposed to the protein before capture of the analyte by the tethered ligand surface.

30. (Withdrawn) The method of claim 21, wherein the detection of the captured analyte is accomplished through the radioactivity of a reactive compound exposed to the protein after capture by the tethered ligand surface.

31. (Withdrawn) The method of claim 21, wherein the detection of the captured analyte is accomplished through the luminescence of a reactive dye conjugate exposed to the protein before capture of the analyte by the tethered ligand surface.

32. (Withdrawn) The method of claim 21, wherein the detection of the captured analyte is accomplished through the luminescence of a reactive dye conjugate exposed to the protein after capture of the analyte by the tethered ligand surface.

33. (Withdrawn) The method of claim 21, wherein the detection of the captured analyte is accomplished through the phosphorescence of a reactive dye conjugate exposed to the protein before capture of the analyte by the tethered ligand surface.

34. (Withdrawn) The method of claim 21, wherein the detection of the captured analyte is accomplished through the phosphorescence of a reactive dye conjugate exposed to the protein after capture of the analyte by the tethered ligand surface.

35. (Withdrawn) The method of claim 21, wherein the detection of the captured analyte is accomplished through the optical absorbance of a reactive dye conjugate exposed to the protein before capture of the analyte by the tethered ligand surface.

36. (Withdrawn) The method of claim 21, wherein the detection of the captured analyte is accomplished through the optical absorbance of a reactive dye conjugate exposed to the sample after capture of the analyte by the tethered ligand surface.

37. (Withdrawn) The method of claim 21, wherein the detection of the captured analyte is accomplished through the fluorescent quenching of the fluorescent tethered ligand surface upon binding of the protein.

38. (Cancelled)

39. (Cancelled)

40. (Cancelled)

41. (Cancelled)

42. (Cancelled)

43. (Cancelled)

44. (Cancelled)

45. (Cancelled)

46. (Cancelled)

47. (Cancelled)

48. (Cancelled)

49. (Cancelled)

50. (Cancelled)

51. (Cancelled)

52. (Cancelled)

53. (Currently Amended) ~~The method of claim 51, wherein the ligands utilized in the array are tethered with a photostable linker at a distance of at least six Å from the substrate surface for the capture of proteinaceous toxins.~~ A method for identification of a protein analyte ~~(proteinaceous toxin or cytosolic protein)~~ comprising:

(a) exposing a solution containing the protein analyte to an array of different peptide ligands which have been covalently tethered with a ~~photostabile~~ photostable linker to a substrate surface at a distance of at least six Å from the substrate surface;

(b) separating the bound protein analyte on the ligand array from the non-binding components of the solution by physical separation of the substrate surface from the sample, washing or both; and

(c) interrogating the ligand-tethered substrate surface with a detection method capable of detecting the bound analyte for protein analyte binding through the:

(i) intrinsic fluorescence of a tryptophan and/or tyrosine residue present in the captured protein where said intrinsic fluorescence is detected between 300 and 400 nm upon excitation by ultraviolet light between 200 and 300 nm;

(ii) fluorescence of a reactive dye conjugate exposed to the protein after capture of the analyte by the tethered ligand surface;

(iii) radioactivity of a reactive compound exposed to the protein after capture by the tethered ligand surface;

(iv) luminescence of a reactive dye conjugate exposed to the protein after capture of the analyte by the tethered ligand surface;

(v) phosphorescence of a reactive dye conjugate exposed to the protein after capture of the analyte by the tethered ligand surface;

(vi) optical absorbance of a reactive dye conjugate exposed to the sample after capture of the analyte by the tethered ligand surface;

(vii) the fluorescent quenching of the fluorescent tethered ligand surface upon binding of the protein.

(d) wherein the protein analyte is selected from the group consisting of:

(i) a proteinaceous toxin

(ii) a cytosolic protein; and

(iii) a proteinaceous hormone.

54. (Cancelled)

55. (Cancelled)

56. (Cancelled)

57. (Cancelled)

58. (Cancelled)

59. (Cancelled)

60. (Cancelled)

61. (Cancelled)

62. (Cancelled)

63. (Cancelled)

64. (Cancelled)

65. (Cancelled)

66. (Cancelled)

67. (Cancelled)

68. (Cancelled)

69. (Cancelled)

70. (Cancelled)

71. (Cancelled)

72. (Cancelled)

73. (Cancelled)

74. (Cancelled)

75. (Cancelled)

76. (Cancelled)

77. (Cancelled)

78. (Cancelled)

79. (Cancelled)

80. (Cancelled)

81. (Cancelled)

82. (Cancelled)

83. (Cancelled)

STATUS OF AMENDMENTS

A Proposed Amendment mailed April 22, 2005 was rejected by the Examiner in an advisory action dated May 13, 2005.

SUMMARY OF INVENTION

The present invention describes a method for the binding of pathogenic microorganisms and their toxic proteins with ligands that have been covalently tethered at some distance from the surface of a substrate: distances of at least fifteen Å (but preferably around forty Å) are required for microorganisms binding ligand tethers and at least six Å are required for protein binding ligand tethers. The ligands described herein include heme compounds, siderophores, polysaccharides, and peptides specific for toxic proteins, outer membrane proteins and conjugated lipids. Non-binding components of

the solution to be analyzed are separated from the bound fraction, and binding is confirmed by detection of the analyte via microscopy, fluorescence, epifluorescence, luminescence, phosphorescence, radioactivity, and optical absorbance. By patterning numerous ligands in an array on a substrate surface it is possible to taxonomically identify the microorganism by analysis of the binding pattern of the sample to the array.

The claims of the divisional patent application in question center on the capture of proteinaceous material via tethered peptide ligands, their separation from non-binding components and the detection of the captured ligands through various methods disclosed in the specification.

ISSUES

In the final rejection, the examiner cites "new ground(s) of rejection . . . made in view of Powers (WO 98/49557) in view of Hudson, et al. (5,576,220)." This new rejection is based on the examiner's conclusion that "it would have been obvious to one having ordinary skill in the art to include in the method of Powers, et al., a physical separation of the bound analyte from non-binding components as taught by Hudson, et al., in order to prevent signal removal by extensive washing." Although Hudson, et al. teaches against using the method of either Powers, et al. or the instant application, the novelty of the application lies in the teachings of the requirements of the linker length covalently tethered to a substrate surface or having a length of at least 6 Å, and not in the steps of physical separation and/or washing.

GROUPING OF CLAIMS

Non Applicable.

ARGUMENT

It is our position that Powers, et al. (WO 98/49557) teach a method for the identification of a biological analyte comprising (pg. 25, first paragraph): (a) exposing a solution containing the analyte to a ligand specific for the analyte of interest that has been tethered to a sensor chip surface, and (b) subjecting the ligand-tethered substrate surface with electromagnetic radiation to detect analyte binding via ratio fluorescence (copending application 659,043). However, Powers, et al. do not teach the use of photostable linkers. The instant application teaches, "It is important to note that the tether should not be photocleavable or otherwise chemically labile in the solution used to wash the ligand-tethered surface." (pg. 11, paragraph 2 – also mentioned on pg. 8, paragraph 1). Powers also fails to teach both the separation of the bound analyte from the sample solution and (most importantly) the importance of the tether length between the ligand and the substrate surface.

As cited by the examiner, both Powers, et al. and the instant application use identical chemistries as illustrative examples of how to tether ligands to substrate surfaces. However, both Powers, et al. (pg. 19, paragraph 2) and the instant application (pg. 20, paragraph 2) teach, "The various ligands are preferably tethered to a substrate by means of organic coupling agents which are themselves known to those skilled in the art." The instant application goes further (pg. 21, last paragraph – pg. 22, first paragraph) when it states, "The chemical reactions used in tethering ligands to the surface of the sensor chip are known to those skilled in the art and are described in the literature. Such reactions may be found in G. T. Hermanson Bioconjugate Techniques (San Diego: Academic Press, 1966); Hansson, et al., "Carbohydrate-Specific Adhesion of Bacteria to Thin Layer Chromatograms: A Rationalized Approach to the Study of Host Cell

Glycolipid Receptors" (Analytical Biochemistry 146: 158-163 (1985)); and, Nilsson, et al., "A Carbohydrate Biosensor Surface for the Detection of Uropathogenic Bacteria" (Bio/Technology 12: 1376-1378 (December 1994))." Powers, et al. further teach (pg. 23, paragraph 2), "As will be appreciated by those skilled in the art, many other techniques can likewise be used to tether an appropriate ligand to the surface. . . ." Similarities between the tethering chemistries (which are known to those skilled in the art) in Powers, et al. and the instant application are present; but it is important, however, to note the differences in what each specification teaches about the tethers themselves.

As cited by the examiner, and most importantly, Powers, et al. do not teach the linker being covalently tethered to a substrate surface via a tether having a length of at least 6 Å. This is a **notable** difference between Powers, et al. and the instant application, which teaches not only the required length of the tether, but the reason why the tether size is important (pg. 11, paragraph 2): "***Binding efficiency is dependent upon the length of the tether.*** Microbes are found to bind most efficiently to ligands that are around forty Å long. Ligands directed to microbes are covalently attached to the substrate surface by tethers that are at least fifteen Å in length; ligands directed to proteinaceous toxins are at least six Å long."

At issue, and central to the rejection by the examiner, is that Hudson, et al. teach separating weak binding and excess analyte from bound analyte by physical separation (e.g., centrifugation Column 18, lines 1-24) or washing (Column 14, lines 37-42). However, the washing and/or physical separation taught by Hudson is for removal of weak binding components of the solution from the HPMP lawyer, not for the removal of non-binding components of a solution from a surface. The presence of the HPMP layer is fundamental to the invention of Hudson, et al. The HPMP layer is disclosed as necessary

to (1) overcome surface effects on tagged target molecule (TTM)-ligand interactions (col. 3, lines 5-8), (2) permit rapid diffusion of unbound molecules from the HPMP matrix back into the bulk solvent (col. 3, lines 52-55), (3) provide a hydrated (solvent-like) environment for ligand binding (col. 2, lines 13-21), and (4) increase ligand densities (col. 4, lines 56-62). The introduction of the HPMP layer to the substrate surface provides a "tortured path" for the analyte solution and introduces a partition thereby increasing nonspecific adsorption.[1] The HPMP layer, much like a gel or particles used in affinity chromatography, slows the flow of the TTM-containing solution, thereby increasing residence time of the analyte in the vicinity of the ligand. This increase in exposure time of the TTM to the ligand improves binding kinetics. It will be appreciated by those skilled in the art that any weak-binding or excess TTMs would still be dissolved in the solution contained in the HPMP. Since the HPMP was designed to remain hydrated (col. 3, lines 52-55), the excess and weak-binding TTMs would remain in the HPMP and need to be removed before detection of the tags. Thus, one reading Hudson, et al. would appreciate that removal of excess and weak-binding TTMs was occurring from the HPMP.

Additionally, Hudson, et al. teach that washing can remove signal arising from specifically bound material, stating (col. 16, lines 57-58), "The more extensive the washing the more signal is removed." However, Hudson, et al. counsel against using surface-tethered peptide ligands (with consequential low aqueous solubility and detrimental surface interactions) as close to the surface as the present application teaches

[1] The effects of the matrix on soluble analytes are known to those reasonably skilled in the art. The partition process is discussed in detail on pg. 18 in "The Principles of Thin-Layer Chromatography" by Kurt Randerath (1963, Academic Press, New York). The equilibria between analyte and matrix are described on pp. 7-11 in "An Introduction to Chromatography on Impregnated Glass Fiber" by Frederick Haer (1968), Ann Arbor Science Publishers, Inc., SBN 87591-007-6).

due to artifacts introduced by surface effects (col. 3, lines 5-8). Subsequently, having knowledge of Hudson, et al., and of the negative impact washing would have on the strength of the signal arising from analyte-ligand binding that it teaches (especially when the ligand is tethered to the substrate surface), it would be apparent that washing would be detrimental. ***Thus, one reading Hudson would be counseled not to use either the method of the instant application or of Powers, et al.***

Additionally, there is another important difference between Hudson, et al. and the instant specification as to the reason washing is used. This additional difference arises from the differing applications of the technologies and methods. Hudson, et al. teach washing in order to reuse ligand-conjugated HPMP surfaces (Abstract and Column 8, lines 17-19), and for "removal of excess target and other molecules" (Column 3, lines 60-63). The instant application teaches (pg. 12, paragraph 3), "Physical separation and washing remove non-binding components of the solution." It can be appreciated by those skilled in the art that if "excess target" (i.e., more target in solution than surface-tethered ligands) were present in the method taught by the instant application, then quantitation of analyte (like that taught in Figure 1 of the instant application) would not be possible since ligand saturation would occur. Furthermore, the method taught by Hudson, et al. is designed to provide "an environment substantially equivalent to natural aqueous solutions for affinity binding" (Column 3, lines 66-67), thus addressing ligand solubility issues (col. 3, lines 2-5), and target analyte elution considerations (col. 4, lines 63-65) that they teach would be present in the method described in the instant application. The method described by Hudson, et al. thus allows weak affinity binding between analytes and ligands to be determined (col. 18, lines 1-9) by using excess target analyte. Using an excess of target is both known and practiced by those skilled in the art in determining

binding constants between ligands and biological analytes – see Chapter 2 of Winzonr D.J. & Sawyer, W.H. Quantitative Characterization of Ligand Binding (New York: Wiley-Liss, 1995).

Hudson, et al. teach (Column 14, lines 34-38), "The winks [HPMP ligand-tethered surfaces] were suspended in [0.15 mL] of phosphate buffered saline, and various concentrations of ^{125}I-radiolabeled Streptavidin were added and *incubated for ca. 2 hours*. The winks were washed and counted on a gamma counter to determine binding saturation." The instant application teaches (pg. 3, paragraph 2), "Thus, there is clearly a need for rapid and inexpensive techniques to conduct field assays for toxic proteins and pathogenic microorganisms that plague animals as well as humans", and (pg. 6, paragraph 1), "In addition, such techniques require high sensitivity when less than 100 cells are present and analysis that can be completed in the field in *less than 15 minutes*." Thus, the differences in purpose between Hudson, et al. (determination of solution-estimated equilibrium binding) and the instant application (rapid determination and quantitation of biological analytes) are illustrated.

CONCLUSION

For the reasons outlined above claims 21-23, 26 and 53 should be allowed.

Respectfully submitted,

September 21, 2005

K. S. Cornaby
Attorney for Applicants
Jones Waldo Holbrook & McDonough PC
170 South Main Street, Suite 1500
Salt Lake City, UT 84101
(801) 521-3200

APPENDIX A

The following 5 claims in the application are appealed.

21. (Currently Amended) A method for ~~taxonomic~~ identification of a biological analyte comprising:

(a) exposing a solution containing the analyte to a ligand specific for the analyte of interest that has been covalently ~~tethered~~ bound directly to a photostable linker, said linker covalently tethered to a substrate surface wherein said photostable linker has a length of at least 6 Å, ~~to a substrate surface with a photostable linker at a distance of at least six Å for the capture of proteins~~;

(b) separating the bound analyte from the non-binding components of the solution containing the analyte by physical ~~separation,~~ separation of the substrate surface from the sample, washing or both; and

(c) interrogation of the ligand-tethered substrate surface for analyte binding by detection of the bound analyte.

22. (Original) The method of claim 21, wherein the biological analyte is selected from the group comprised of:

(b) proteinaceous toxins; and

(c) cytosolic proteins.

23. (Currently Amended) The method of claim 21, wherein the ligand is a peptide, ~~usually~~ comprised of three to twenty amino acids ~~long~~, specific for a proteinaceous toxin.

26. (Currently Amended) The method of claim 21, wherein the ligand is a peptide ~~that does not contain tryptophan or tyrosine~~ and detection of the captured analyte is accomplished through interrogation of the surface to detect ~~the~~ an intrinsic fluorescence of the ~~protein.~~ tryptophan and/or tyrosine residues present in the captured protein where said intrinsic fluorescence is detected between 300 and 400 nm upon excitation by ultraviolet light between 200 and 300 nm.

53. (Currently Amended) ~~The method of claim 51, wherein the ligands utilized in the array are tethered with a photostable linker at a distance of at least six Å from the substrate surface for the capture of proteinaceous toxins.~~ A method for identification of a protein analyte ~~(proteinaceous toxin or cytosolic protein)~~ comprising:

(a) exposing a solution containing the protein analyte to an array of different peptide ligands which have been covalently tethered with a ~~photostabile~~ photostable linker to a substrate surface at a distance of at least six Å from the substrate surface;

(b) separating the bound protein analyte on the ligand array from the non-binding components of the solution by physical separation of the substrate surface from the sample, washing or both; and

(c) interrogating the ligand-tethered substrate surface with a detection method capable of detecting the bound analyte for protein analyte binding through the:

(i) intrinsic fluorescence of a tryptophan and/or tyrosine residue present in the captured protein where said intrinsic fluorescence is detected between 300 and 400 nm upon excitation by ultraviolet light between 200 and 300 nm;

(ii) fluorescence of a reactive dye conjugate exposed to the protein after capture of the analyte by the tethered ligand surface;

(iii) radioactivity of a reactive compound exposed to the protein after capture by the tethered ligand surface;

(iv) luminescence of a reactive dye conjugate exposed to the protein after capture of the analyte by the tethered ligand surface;

(v) phosphorescence of a reactive dye conjugate exposed to the protein after capture of the analyte by the tethered ligand surface;

(vi) optical absorbance of a reactive dye conjugate exposed to the sample after capture of the analyte by the tethered ligand surface;

(vii) the fluorescent quenching of the fluorescent tethered ligand surface upon binding of the protein.

(d) wherein the protein analyte is selected from the group consisting of:

(i) a proteinaceous toxin

(ii) a cytosolic protein; and

(iii) a proteinaceous hormone.